Fluorescence modulation of long DNA molecules adsorbed onto a microelectrode surface

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(Received 2 July 2003; accepted 20 October 2003)

Using microfabricated electrodes, we demonstrate fluorescence modulation of deoxyribonucleic acid (DNA) stained with dye through the application of cyclic electric fields. The fluorescence of DNA adsorbed onto an electrode was quenched under the potentials greater than about 1 V, and was recovered by reducing the potentials to below about 0.6 V. The quenching was observed even for a single DNA molecule adsorbed on an electrode. Therefore, the fluorescence modulation of DNA by external electric fields can be expected as one of selective detection methods for adsorbed DNA onto electrodes. © *2003 American Institute of Physics.* [DOI: 10.1063/1.1633333]

Long DNA molecules tend to adsorb evenly onto low-reactive metal surfaces, such as silver, gold, and platinum, especially in the presence of electric fields.[1,2] Although many electrochemical studies involving DNA-modified electrodes have been performed,[3–5] a detailed understanding of the optical and electrochemical properties of DNA–metal interactions is lacking. In this study, we investigated the fluorescence emission and quenching of DNA-dye complexes adsorbed onto gold electrodes by simultaneous application of fluorescence measurement and cyclic voltammetry. Our results demonstrated that DNA adsorption increases the redox current, and that the transition from a continuous emission state to a quenched state is induced by the regulation of the electric field. We think about the possible electrochemical detection of a single DNA molecule based on the fluorescence modulation.

Experimental setup is similar to that in our previous work.[1] A pair of Au electrodes {100 μm width, 5000 μm length [see Figs. 1, 2(a) and 2(b), and 4], 50 μm width, 500 μm length (see Fig. 3), Au: ~80 nm thickness, Cr: ~20 nm thickness} was fabricated on a quartz plate (72 mm×26 mm). A 15 μl sample solution was dropped into the electrode gap and then sealed with a cover slip (22 mm×22 mm). Cyclic electric potentials were then applied by a function generator and a potentiostat. Fluorescence intensity of the DNA dye was monitored using a photomultiplier tube (PMT). Current, voltage, and fluorescence were simultaneously recorded using a data recorder.

Prior to PMT measurement, T4 DNA (166-kb, Nippon Gene, Inc., Japan) stained by YOYO-1 (Molecular Probes, Inc., OR, USA) was immobilized on a gold electrode in 0.5× TBE buffer solution (pH 8.0, 45 mM tris-borate, 1.25 mM EDTA).[1] Figure 1(a) shows the fluorescence image before voltage application, and it shows all DNA is free, and not immobilized. The four lines indicate the edges of the electrodes with a 20 μm gap and 100 μm width. We used a two-electrode system for simplicity. The upper electrode is the working electrode (WE), the lower the counterelectrode (CE). Most DNA molecules were immobilized on the anode surface (WE) after application of a potential of 2 V for a few tens of seconds [Fig. 1(b)].[1] The area lit by an excitation mercury lamp was then limited to a circle of about 100 μm diameter on the WE [Fig. 1(c)]. After checking for fluorescence quench [Fig. 1(d)] and recovery [Fig. 1(e)], 3-CCD camera was replaced by the PMT. The PMT then monitored the fluorescence from 50 to 100 DNA molecules adsorbed to the lit area.

Figure 2(a) shows time courses for the applied triangle potential (thick line), current (thin line), and fluorescence (dotted line). The potential was swept with 12.5 mV/s, and the amplitude was changed at 42 s from 0-2 to 0-1 V. While the DNA was exposed to the voltage varying up to 2 V, the fluorescence was modulated in synchronization with the external field (region 1). When the maximum voltage was switched to 1 V at 42 s, the fluorescence shifted to a steady state (region 3) via a transient state (region 2). In region 3, the DNA had an essentially constant emission (about 4 F.U.) apart from asymptotic fading of the fluorescence due to the continuous lightning.

Figure 2(b) shows current–voltage ($C-V$, solid black line) and fluorescence–voltage ($F-V$, dotted black line and solid gray line) curves of DNA-YOYO1 complexes in 0.5× TBE solution. Each curve rotates clockwise as indicated by arrows. $C-V$ curve of a gray line is for a 0.5× TBE only. The increasing of current in the presence of DNA is also

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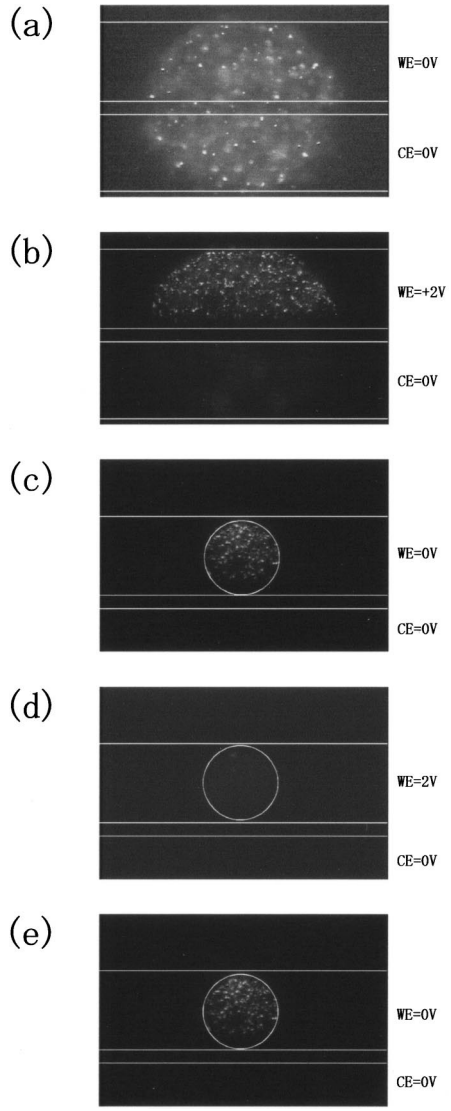

FIG. 1. Fluorescent images of T4 DNA molecules around the Au microelectrodes: (a) equilibrium state; (b) preliminary voltage application of +2 V (anode is upper); (c) the excitation area focused on the WE, 0 V; (d) quenching, +2 V; (e) reemission, 0 V.

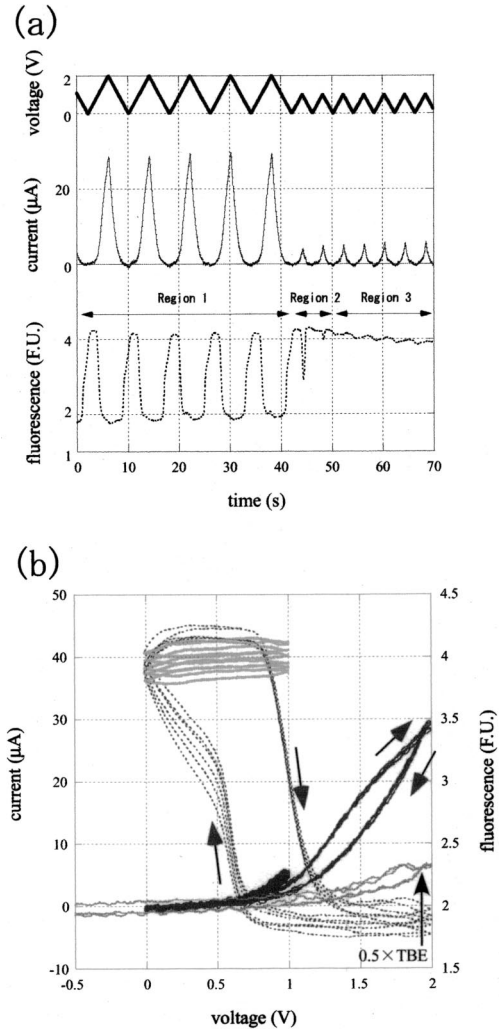

FIG. 2. (a) Time courses of voltage (thick), current (thin), and fluorescence (dotted). (b) cyclic voltammogram (dotted: 2 V, gray: 1 V) and fluorescence intensity (thin: 2 V, thick: 1 V). The gray line indicates 0.5× TBE buffer only and other lines indicate 0.5× TBE buffer containing T4 DNA.

caused by DNA adsorption. The $C-V$ with DNA deviates from that in the 0.5× TBE in the region more than 1 V. Accompanying this current increasing, fluorescence quenching occurs from the region where voltage exceeds about 1 V. This implies that fluorescence quenching is coupled with electron transfer at the electrode surface. On the other hand, the recovery of emission starts at around 0.6 V. This hysteresis in the threshold voltages makes the $F-V$ curve in the modulated state to be a large loop. In a steady state, both $F-V$ (gray line) and $C-V$ (thick black line) curves trace the $F-V$ and $C-V$ for the modulated state part of the way. Therefore, the threshold value exists in the external voltage between 1 and 2 V.

Figure 3 shows plots of relative fluorescence intensity versus current. The applied voltages are indicated on the left of each figure. Fluorescence intensities are normalized by each maximum of the fluorescence data. Under 1 and 1.2 V, fluorescence is steady emission state. On the other hand, under 1.6, 1.8, and 2 V, fluorescence is modulated between a fully quenched state and an emission state. Around 1.4 V, the

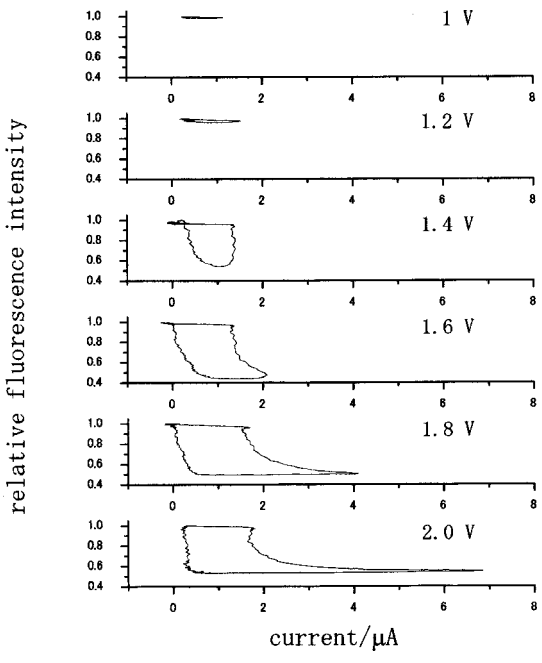

FIG. 3. Plots of relative fluorescence intensity vs current in the range from 1 to 2 V. Fluorescence intensities are normalized by each maximum of the fluorescence data.

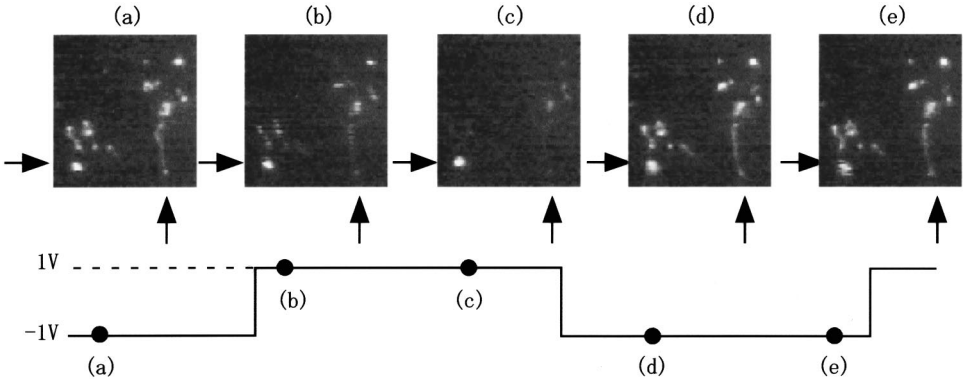

FIG. 4. Sequential fluorescent images of T4 DNA molecules around Au microelectrodes under a rectangle potential (2 Vpp, 3 Hz).

quench level of fluorescence is not completed. The fact indicates that the region is a transient region. This indicates that the transition from a continuous emission state to a quenched state is not abrupt but diffuse for this kind of a many molecules system.

Because of the continuous property as shown in Fig. 3, rectangle potential is preferable to triangle potential for usage of the fluorescence modulation in molecular detection. Figure 4 shows sequential fluorescence images of T4 DNA at an Au electrode under a rectangle potential (2 Vpp, 3 Hz). The potential levels are indicated on the schematic below the images. Images (a)–(e) were taken at 0.066 s intervals. The horizontal arrows in each image indicate a free excess DNA molecule, which could not adsorb on the electrode under rather high frequency field conditions. The fluorescence was not quenched at any of the potential levels. In contrast, fluorescence of DNA molecules immobilized onto an electrode (as indicated by the vertical arrows) was quenched at $+1$ V [(b) and (c)], but recovered at -1 V [(d) and (e)]. Therefore, fluorescence modulation can be observed at the single DNA molecule level.

Thus, by simultaneous monitoring of fluorescence and current, the adsorption of a single DNA molecule onto an electrode could be detected selectively. We aim to apply this method to the single DNA molecule detection and identification in microfluidics devices. Further investigation using smaller electrodes is required and will be undertaken in future work.

This work was partially supported by a Grant of Core Research for Evolutional Science and Technology (CREST) from the Japan Science and Technology Corporation (JST), The Murata Science Foundation.

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